

JOURNAL OF AGRICULTURAL RESEARCH

VOL. IX

WASHINGTON, D. C., APRIL 2, 1917

NO. 1

A NEW BACTERIAL CITRUS DISEASE¹

By H. AHERTON LEE,²

*Scientific Assistant, Division of Plant Pathology, University of California
Agricultural Experiment Station*

INTRODUCTION

During the last few years a disease of Citrus trees, and particularly of orange trees, has been brought repeatedly to the attention of different members of the Agricultural Experiment Station staff of the University of California. In some respects the trouble resembles frost injury, so that many growers, believing that it was due to frost, did not appreciate its full significance. Occasionally material has been brought to the laboratories of plant pathology at Berkeley and at Riverside; but such material has been old and dried and has given no clue to the real cause.

In March, 1916, Dr. J. E. Coit, of the division of citriculture, called attention to this disease and discussed its seriousness.³ Since he found no similar disease described in literature, he called it "Citrus blast," a new disease.

DESCRIPTION OF THE DISEASE

During the rainy season of California, usually about the middle of January, the disease is first noticed. Young leaves are found to be dropping off, sometimes leaving single twigs or at other times whole branches of twigs bare of leaves. On examining more closely, black, discolored areas are noted on the leaves; such areas are found most commonly at the junction of the leaf blade and the wings of the petiole. Plate A, figure 3, shows such lesions. The affected parts have a water-soaked appearance, and the whole leaf loses its rigidity and hangs limply from the branch. Less commonly such water-soaked lesions appear near the tips of the leaves.

¹ Approved for publication by Thomas F. Hunt, Director, California Agricultural Experiment Station.

² Now Scientific Assistant, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture.

The author wishes to express appreciation and thanks to Prof. Ralph E. Smith, of the University of California, for direction of this work; also to Dr. William A. Setchell, of the same university, for the translation into Latin of the description of the organism.

³ Coit, J. E. Citrus blast—a new disease in California. *In Univ. Cal. Jour. Agr.*, v. 3, no. 6, p. 234-235, illus. 1916.

The blackened areas frequently spread down the petioles of the leaves into the twigs upon which the leaves are borne. If such a twig is young and actively growing, the diseased area spreads quickly and the whole twig becomes blackened and shriveled. In young orchards, where there is much new succulent growth, these blackened, shriveled twigs, bare of leaves, are very common; and in this condition there is little similarity between such affected parts and frost injuries. The disease has never been found spreading down into the mature wood.

In March there are periods of several days in which the weather becomes very warm and the orchards dry up to some extent. Such weather causes the affected tissues at the base of the leaf blade and the affected leaf petioles to dry up, become stiff and rigid, and the leaves to hang to the tree. The trees in such cases resemble pear trees badly affected with pearblight in the young succulent twigs (Pl. 2).

In many cases the lesion spreads from the petiole of a leaf down into twig tissue which is still soft but not actively growing, and a small, black area around the base of the petiole is formed. After the leaf has dropped off and the dry season comes on, brown blister-like scabs are formed over such affected areas (Pl. A, 1, 2). As the twig continues growing, these scabs become loose and may be cast off during the succeeding fall. On trees which have been affected in the spring, however, such blisters are frequently found in the following winter, and these are presumably the sources for the dissemination of the disease.

DISTRIBUTION OF THE DISEASE

The disease was first observed in 1912 at Oroville, Cal., by Mr. R. W. Hodgson, of the University of California. Since then specimens have been received from time to time from the Oroville and Orland regions, and it has now been seen or reported in almost all the Citrus-growing regions of northern and central California, both on the orange (*Citrus aurantium* L.) and the lemon (*Citrus limonum* Risso). It has not been observed in southern California, and, so far as is known, no one has reported it from there as yet.

ISOLATION AND IDENTIFICATION OF THE ORGANISM

On January 24, 1916, fresh material of Citrus blast was sent in from Palermo, Cal. Sections through the lesions were made, and the tissues were seen to be filled with motile bacterial organisms. Isolations made from this fresh material in +10 standard peptone-beef bouillon¹ produced a clouding on the second day; on plating, these yielded several sorts of colonies. One of these types of colonies, on being inoculated into orange trees in the greenhouse, gave positive results con-

¹ All references to acidity are expressed in terms of Fuller's scale.

sistently; and reisolations from these inoculations gave the same typical colonies. The organism has been repeatedly inoculated and reisolated since that time, and has also been repeatedly isolated from material from different regions of the State, both from the fresh black lesions and the reddish blister-like scabs found in summer. Both types of lesions, the black watery effect on the leaves and twigs and the reddish scabs, have been produced from artificial inoculations with pure cultures. In the inoculation work young lemon and navel-orange trees have been used and kept in the greenhouse at temperatures of 20° to 25° C.

According to the methods in use at present by bacteriologists, the organism is a distinct species; and, so far as is known, has never been described in the literature on the subject. Owing to the withering or drying up of the leaves of Citrus trees following the attacks of this organism the specific name "*citrarefaciens*" is suggested—a compound of the two words "Citrus" and "arefacio." The brief Latin diagnosis is as follows:

Bacterium citrarefaciens, sp. nov.

Baculis cylindricis, apicibus rotundatis, solitariis aut interdum geminis, $1.2-3 \times 0.4-0.9 \mu$, vulgo $0.6 \times 1.8 \mu$, motilibus, flagellis 1-4, uni-aut-bipolaribus; methodo Grami non coloratis; cum acidibus decoloratis; sporis capsulisque nondum visis; zoogloeis defectis; statibus involutis, longe-filamentosis, massulas protoplasmaticas densiore tintas completentibus, coloniis in agar-agar orbicularibus, convexis, nitentibus, margaritaceo pallidis, colorem fluorescenti-viridem in medium alibile efficientibus; gelatinis primum celeriter deinde lente liquefacientibus; casein segregantibus; lactem litmus lente decolorantibus; in mediis saccharatis neque gas neque acidum evolunt; nitrum non reducentibus; indol ammoniamque moderate producentibus; aerobicis sed in presentia sacchari, sacchari uvae, aut sacchari hordei facultative anaerobicis. In textis vivis arborum citrinorum, laesiones foliorum cauliumque juvenorum, primum nigras deinde in mensibus siccis aestivalibus rubras scabrosasque efficiens.

HISTOLOGY

Affected areas of young leaves were fixed, embedded, sectioned, and stained. It was found that the organism in tissues could be stained best with Mallory's chlorid-of-iron hematoxylin, as described by Mallory and Wright.¹ Impregnation with gold chlorid, according to Lee,² also gave good results, the impregnation being carried on after the sections had been fixed on the slides, however, instead of *in toto*, as described by Lee.

Masses of bacteria could be observed throughout the parenchyma of the leaf, both in the cells and in the intercellular spaces. In many lesions all cell structures had disappeared, the areas being occupied by masses of the organisms (Pl. 1, C). The disease is not to be considered as an invasion of the vascular bundles, but rather as an attack upon the parenchyma.

¹ Mallory, F. B., and Wright, J. H. *Pathological Technique* . . . ed. 3, 469 p., 138 fig. Philadelphia, 1904.

² Lee, A. B. *Microtome's Vade-Mecum* . . . ed. 7, 526 p., illus. Philadelphia, 1913.

MORPHOLOGY AND PHYSIOLOGY OF BACTERIUM CITRAREFACIENS

MORPHOLOGICAL CHARACTERS

When the organism is taken from 24-hour-old agar stroke cultures it is rod-shaped, with rounded ends, usually single, but occasionally in pairs. The limits of size are 1.2 to 3.0 by 0.3 to 0.9 μ , the most common size being 1.8 by 0.6 μ . No spores or capsules have been observed; zoogloea are not found; motility has been observed in affected tissue and from young cultures, one to four flagella at one or both poles having been shown from 24-hour agar cultures. Involution forms, long filament-like bodies in which there are thickened masses of protoplasm that take stains more heavily, are found in old cultures and especially in saccharose bouillon (Pl. 1, B).

The bacterial rods may be readily stained by watery-fuchsin, gentian-violet, carbol-fuchsin, and Löffler's alkaline methylene-blue. When stained lightly, a bipolar effect is brought out in the organism, the ends of the rods taking the stain more densely, leaving a lighter area in the middle. The organism is Gram-negative, and is not acid-fast.

CULTURAL CHARACTERS

AGAR POURED PLATES.—On +10 peptone-beef agar at 20° to 22° C., surface colonies are apparent on the third day; on the fourth day colonies are 2 to 3 mm. in diameter, white, round, smooth, glistening, convex, finely granular (under the compound microscope), with entire edges sometimes becoming lacerate, due to the semifluid consistency of the bacterial mass. When 5 days old, the agar beneath the colonies becomes greenish and fluorescent, and in 7 days the colonies lose their convex glistening surface and dry down, frequently becoming concentrically ringed and the edges undulate to lobate. Buried colonies are biconvex.

AGAR STABS.—Stabs in +10 peptone-beef agar when 13 days old show growth along the line of inoculation to four-fifths of the total depth of the stab. Growth is best toward the surface, the line of puncture beaded; there is no liquefaction; surface growth is restricted. The agar medium is uniformly colored a javal-green¹ when seen against a black background.

AGAR SLANTS.—On slant agar, stroke cultures make an abundant growth in two days; filiform becoming somewhat echinulate, convex, glistening, smooth, translucent, of slimy consistency, and with a slight odor of putrefaction. A cream-white sediment forms in the V; the medium is colored uniformly a fluorescent-green.

NUTRIENT-GELATIN PLATES.—Growth rapid, at first punctiform, then round, crateriform; liquefaction spreading.

GELATIN STABS.—At 18° to 19° C., in +15 peptone gelatin, liquefaction starts on the first day, is crateriform until on the fourth day the pit of liquefaction reaches the walls of the tubes when it becomes stratiform. The liquefaction gradually slows up and takes 60 days or more to reach completion.

BEEF BOUILLON.—In +10 peptone-beef bouillon a moderate uniform clouding appears within 24 hours; there is no turbidity. At first there is formed a very thin pellicle which disappears within a few days. Old cultures contain a sediment which is very slightly viscid. There is a slight odor of putrefaction.

¹ All references to color are expressed according to Ridgway (Ridgway, Robert, *Color Standards and Color Nomenclature*. 43 p., 53 pl. Washington, D. C., 1922).

POTATO CYLINDERS.—Growth on potato cylinders is moderate, filiform, becoming echinulate, and spreading with age; at first convex, becoming flattened, glistening, smooth, cream-buff in color, the medium itself being slowly browned until it becomes avellaneous. There is a slightly saline odor from the cultured cylinders. At the end of the fifteenth day starch reduction was tested by means of iodine in a potassium-iodid solution. Uninoculated cylinders gave the typical blue starch reaction, but cultured media gave the port-wine color indicative of the formation of amylopectrin or still lower reduction products.

MILK.—The casein in inoculated milk coagulates slowly, leaving at the surface a clear liquid which has a fluorescent-green color. Peptonization of the coagulum then takes place slowly, being complete in 25 to 30 days. The consistency of the medium is unchanged.

LITMUS MILK.—Growth in litmus milk is distinctive; the coagulated portion remains the original lavender color, but the cleared portion becomes a deep glaucous gray. Beneath the upper stratum a layer of light violet-gray appears, while a sediment of pale olive-buff collects at the bottom of the tubes. As the coagulum is slowly peptonized, the sediment and the deep glaucous-gray layer increase in depth; and when the coagulum has entirely disappeared the litmus becomes rapidly reduced. At the end of 25 days the tubes have lost all the litmus color, and are similar in color to peptone-beef bouillon. The blue has sometimes been restored by shaking; but at no stage was there any formation of acid.

USCHINSKY'S SOLUTION.—Growth in Uschinsky's solution is heavy, slightly turbid, with no surface growth; on the fourth day the medium becomes viridine-green. In old cultures there is a very slight sediment, which is slightly viscid.

FERMI'S SOLUTION.—Growth is scanty and uniform, with no surface growth; there is a slight green color similar to that produced in Uschinsky's solution.

COHN'S SOLUTION.—Growth in this medium is scanty, forming only a thin, uniform, scarcely visible clouding.

STARCH JELLY.—Starch jelly was made by adding 1 gm. of potato starch to 10 c. c. of Uschinsky's solution. Growth was moderate; the medium was turned to a fluorescent-green color. On adding an iodine solution to the medium a reddish-brown color was first formed, which on standing several minutes entirely disappeared, indicating the formation of achrondextrin, or even lower reduction products. There is a positive diastasic action.

TOLERATION OF SODIUM CHLORIDE.—Tubes of peptone-beef bouillon acidified to +10 and containing 1.5, 2.5, 3, 4, and 5 per cent of pure sodium chlorid were inoculated from 7-day-old agar slant cultures. Growth was moderate in the 1.5 and 2.5 per cent sodium-chlorid cultures, and was scanty in the 3 per cent cultures. In the cultures containing 4 per cent of sodium chlorid a clouding was formed, scarcely apparent to the eye, while in the 5 per cent cultures growth was entirely inhibited.

BOUILLON OVER CHLOROFORM.—Growth is unrestrained in +10 peptone-beef bouillon tubes to which 2 c. c. of chloroform have been added.

FERMENTATION TUBES.—The tests for gas and acid production were made in fermentation tubes containing neutral peptone-beef-litmus bouillon to which was added 2 per cent of the compound to be tested; these were dextrose, saccharose, maltose, mannite, and glycerin. Growth occurred most abundantly in the tubes containing dextrose and saccharose, but growth was abundant in all the sugar media. A reduction of the litmus took place in all the media, especially rapid in the glucose bouillon, the liquid finally becoming the color of standard peptone-beef bouillon. Neither gas nor acid was formed in the presence of any of the sugars after 15 days. In the presence of dextrose, saccharose, and maltose, clouding could be observed in the closed arms of the fermentation tubes; but in those tubes containing the bouillon with glycerin, mannite, and lactose, growth took place only in the open chambers.

AMMONIA PRODUCTION.—Peptone-beef-bouillon cultures tested at the end of the tenth day, using Nessler's reagent, gave a strong reaction for the presence of ammonia.

REDUCTION OF NITRATES.—Nitrates are not reduced. Five-day-old cultures tested both by the starch potassium-iodid test and by the sulphanilic-acid alphanaphthylamin test gave no color reaction.

PRODUCTION OF INDOL.—Tests made at the end of the tenth day on cultures in Dunham's peptone solution gave a strong indol reaction. The cultures tested with the sodium-nitrite sulphuric-acid reaction gave a strong pink color very quickly, while control tubes treated similarly remained uncolored.

TOLERATION OF ACIDS.—Neutral peptone-beef bouillon, to which were added 0.1, 0.2, 0.3, and 0.4 per cent, respectively, of citric, oxalic, and tartaric acids, was inoculated. After five days a moderate growth was visible in the cultures containing 0.1 per cent of all the acids used. This growth increased and was quite strong at the end of the fifteenth day, but in none of the 0.2, 0.3, or 0.4 per cent strengths of any of the acids was clouding visible, although kept for 30 days.

TOLERATION OF SODIUM HYDROXID.—The organism is quite sensitive to alkali. Peptone-beef bouillon titrated according to Fuller's scale to the following strengths: +35, +30, +20, +15, +10, +5, neutral, -5, -10, -20, and -30, were inoculated from a 24-hour-old +10 bouillon culture. Growth was apparent and vigorous in the +10 and +15 bouillon on the second day, and was also apparent, although scanty in those titrated to neutral, +5, and +20. On the third day tubes of -5 bouillon became slightly clouded and on the eighth day -10 bouillon and +30 bouillon showed a very scanty, scarcely visible clouding. Clouding in the -20 bouillon was not visible until after the tenth day, but on the twentieth day was easily apparent. Growth was never visible in the bouillon titrated to +35 and -30. The best growth took place in the +15 bouillon.

HYDROGEN SULPHID.—Strips of filter paper soaked in a saturated solution of lead acetate were suspended over cultures of the organism in a +10 peptone-beef bouillon. No browning of the paper was visible during five weeks' exposure. A medium of +10 nutrient bouillon containing 0.1 per cent of lead-acetate crystals was also inoculated with the organism. Although growth took place moderately, no precipitate of lead sulphid occurred in five weeks.

METHYLENE-BLUE IN MILK.—There is a very slow reduction of methylene-blue in milk. The organism was inoculated into tubes containing a milk medium to which 4 per cent of a 1 per cent solution of methylene-blue had been added. Slight reduction was visible on the third day, and apparently took place but slightly and very slowly. At the end of 15 to 20 days the original color of the milk had entirely disappeared; the coagulum was dissolved and the whey was a pale cendre green, with an upper layer of dark green. This color did not disappear, although the cultures were kept for five weeks.

SACCHAROSE BOUILLON.—A medium of +10 peptone-beef bouillon containing 5 per cent of saccharose was inoculated. Growth was very vigorous, flocculent, and later formed a flaky, somewhat viscid sediment. In this medium filamentous chains of the organism were found.

AEROBISM.—The organism is aerobic in general; but is facultative anaerobic in the presence of dextrose, saccharose, or maltose. Stab cultures in tubes of +15 gelatin from which the oxygen had been removed by Wright's method¹ showed no growth. Likewise poured plates of +15 gelatin showed no growth underneath sterile cover glasses to exclude the air (Koch's method). However, in the closed arms of fermentation tubes containing glucose, saccharose, or maltose bouillon there is a distinctly visible growth.

GROUP NUMBER.—Following the numerical system of the Society of American Bacteriologists, the group number is 221.333213.

¹ Mallory F. B., and Wright, J. H. Op. cit.

EFFECT OF PHYSICAL CONDITIONS

SENSITIVENESS TO DESICCATION

Drops of 24-hour cultures in +15 peptone-beef bouillon were placed upon sterile cover glasses in sterile petri dishes and kept in a dry dark-room. The organism was not killed after 12, 24, or 36 hours; but no growth was obtained after seven days of drying. On the other hand, although susceptible to drying in the air, old Citrus-blast material which had been kept for four months in the laboratory was found to yield the pathogenic organism. Under this condition, then, the organism is apparently able at least to live over the dry summer months.

SENSITIVENESS TO SUNLIGHT

The Citrus-blast organism is rather sensitive to direct sunlight. Thinly sown agar plates from 10-day-old cultures in Uschinsky's solution and from 24-hour bouillon cultures were exposed bottom up to sunlight in September for 5, 10, 15, 30, 45, and 60 minutes. One-half of each plate was protected from the light by black paper, and in each case the plates were protected from heat by being placed upon ice.

Plates exposed for 30, 45, and 60 minutes showed no growth upon the parts exposed to the sunlight. Plates exposed for 15 minutes showed that 82 per cent were killed by the exposure, and those which survived grew more slowly than those unexposed. Plates exposed for 5 and 10 minutes showed colonies growing uniformly on the exposed as well as the unexposed sides.

TEMPERATURE RELATIONS

The best growth obtained was at temperatures between 25° and 28° C. Scanty growth was observed at 16° and at 37° C.

The thermal death point determined by exposing newly inoculated peptone beef-bouillon cultures for 10 minutes to definite temperatures was found to be 50° C. In this case transfers were made from a 24-hour-old culture in peptone-beef bouillon to the bouillon tubes to be tested.

ECONOMIC IMPORTANCE OF CITRUS BLAST

The chief injury caused by Citrus blast is in the killing of the young wood which should bear the blossoms and later the crop. Damage also results from defoliation and a consequent loss of leaf area for the whole tree. The whole result is a decrease in the ability of the tree to produce crops. The disease has also been observed in nurseries, and, although there is no means of knowing the actual loss suffered by this industry, the idea of dissemination by nursery stock immediately suggests itself.

As yet the disease has not caused any widespread damage, but the possibility of its becoming distributed throughout the State and perhaps other Citrus-growing regions is not to be disregarded.

SUMMARY

(1) A new disease of Citrus trees is endemic to the Citrus regions of northern and central California. It has been described and given the name "Citrus blast" by Dr. J. E. Coit, of the University of California.

(2) Sections of fresh disease material show a bacterial organism present in masses, and on isolation plates bacterial colonies are obtained which on inoculation produce the typical lesions of the disease. From such positive inoculations the organism has been reisolated and reinoculated, giving positive results again, and finally being reisolated. The organism is apparently a new species, and the name "*Bacterium citrarejaciens*" is proposed.

(3) The organism exists in the parenchyma and destroys cell structure, leaving large pockets filled with bacterial masses. The organism does not ordinarily invade the vascular bundles and is apparently restricted to the parenchyma.

(4) The disease causes a decrease in leaf surface and a loss of the fruit-bearing wood in orchard trees. Young trees in nurseries may also be injured. The possibility of the disease becoming distributed throughout all California and other Citrus-growing regions is to be considered.

PLATE A

- 1, 2.—Orange twigs, showing brown blister-like scabs of Citrus blast, formed in summer over affected parts.
- 3.—Natural infection upon young leaves of a navel-orange tree.



PLATE I

A.—Flagella of *Bacterium citrarefaciens* from 24-hour-old agar slant. Stained by Löffler's method. $\times 1,000$.

B.—Filamentous bodies showing thickened areas of denser protoplasm from saccharose bouillon cultures of *Bact. citrarefaciens*. Stained with carbol-fuchsin. $\times 450$.

C.—Cross section of a navel-orange leaf affected with Citrus blast. Stained by Mallory's ferric-chlorid hematoxylin method. A few individuals and several masses of *Bact. citrarefaciens* may be observed.

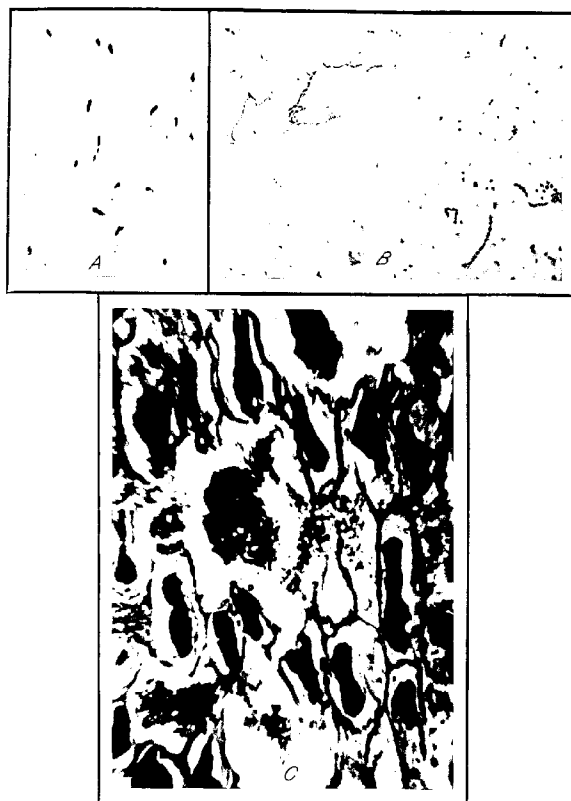




PLATE 2

Tree affected with Citrus blast, showing bared twigs and wilted leaves.

SOME FACTS ABOUT ABORTION DISEASE

By E. C. SCHROEDER, *Superintendent*, and W. E. COTTON, *Assistant*, Bethesda (Md.)
Experiment Station, Bureau of Animal Industry, United States Department of Agriculture

CHARACTERISTICS OF THE ABORTION BACILLUS

While most bacterial diseases have two prime or main factors, a pathogenic microparasite and a susceptible host, infectious abortion disease of cattle is more complex, in that it has three prime factors, a pathogenic microparasite and two hosts. How imperfect our knowledge about this perplexing evil has remained at once becomes apparent when we consider that it has not been certainly determined which of the two hosts, the cow or the fetus, is primarily attacked by the microparasite. That is to say, we do not know whether the abortion bacillus primarily causes a disease of the cow's uterus which leads to the expulsion of the fetus, or whether, in the first place, it causes a disease of the fetus which subsequently impels the uterus to expel its contents.

One of the superlatively important facts about abortion disease is that cows often remain carriers of abortion bacilli long after they have ceased to abort, and that cows which have never aborted and regularly and normally produce seemingly healthy calves may be chronic carriers and disseminators of abortion bacilli.

As far as the writers have been able to learn, the abortion bacillus is an obligatory parasite. It may live and retain its virulence for a long time in infected material expelled from the uteri of infected cows, as such period of time can be measured through bacteriological cultivation and guinea-pig inoculation tests; but no data are available to support the belief that it can maintain itself or multiply under natural conditions as a saprophyte. Hence, the chronic persistence of the microparasite in the bodies of infected cows probably is the most important among the causes responsible for the propagation, the perpetuation, and wide prevalence of the disease.

The favorite habitat of the abortion bacillus in the bodies of cows is the udder, and the udder is seemingly its only habitat in the bodies of nonpregnant cows. Our work regarding this fact includes hundreds of carefully made tests with milk from numerous cows. Some of the cows had aborted, and others had not; the milk of some was infected with abortion bacilli continuously, and that of others intermittently; that of some cows remained infected year after year and that of others for shorter periods of time. In one case (a cow that remained under observation for seven years) periodic tests proved the milk to be infected continuously.

Another fact related to the expulsion of abortion bacilli with milk from the udders of cows is that in the numerous tests made with milk from many different cows the abortion bacillus was never found in the milk of a cow unless both her milk and her blood serum possessed agglutinating properties for suspensions of abortion bacilli. This fact is interesting and important not only on its own account but also because it serves as strong circumstantial evidence to prove that the work of the writers on the occurrence of abortion bacilli in the milk of cows is trustworthy. It does not mean, however, that the milk of all cows which react with the agglutination test for abortion disease is infected, as the writers have repeatedly tested milk from reacting cows without detecting abortion bacilli.

Regarding reacting cows with uninfected udders, it appears that their blood serum gradually loses its power to agglutinate suspensions of abortion bacilli. The writers wish, however, to have this statement taken cautiously, as the evidence behind it is not yet sufficient to give it the rank of a proved fact. If this statement, on further study, should prove true, it, together with other facts, will justify the conclusion that the persistence of agglutinating and complement-fixing substances in the blood of cows, relative to abortion disease, is intimately associated with the abortion bacilli that enter the body through the lymphatics from infected udders, as abortion bacilli do not maintain themselves in the bodies of cows elsewhere than their udders and gravid uteri.

That abortion bacilli do not maintain themselves in the bodies of cows elsewhere than the regions named is a fact of which the writers have obtained fairly convincing proof. It was found that abortion bacilli injected into the veins of normal, nonpregnant cows disappeared from their circulating blood in the course of a few hours; and when such cows were killed sometime afterwards, though their blood had become positive with agglutination tests, the germs could not be found in their bodies unless it was in their udders and associated lymph glands. One case in the records of experiments is remarkably impressive as an illustration of the tendency of abortion bacilli to lodge in the udder. The case is that of an adult, virgin, female animal, a heifer, approximately 4 years old, which was given an injection of abortion bacilli into one of her jugular veins. Later it was found that the infection¹ had established itself in her virgin udder, which was not functioning and never had functioned.

Another series of tests, probably even more convincing than the foregoing, was a careful search for abortion bacilli in the bodies of naturally infected as distinct from artificially infected cows. The cows were killed and their blood, spleens, livers, kidneys, brains, ovaries, uteri, udders, milk, synovial fluid from various joints, nerve tissue, lymph glands from all portions of the body, etc. tested for abortion bacilli

¹ The term "infection" is used here and elsewhere in this paper as signifying the discoverable presence of abortion bacilli, and not as implying the development of observable lesions of disease.

through animal inoculation and cultural methods, with the following results: In all cases two or more quarters of the udder, the milk from the infected quarters, and one or more supramammary lymph glands, and in one instance some of the pelvic lymph glands, were infected. All other organs and tissues were invariably free from infection.

When abortion bacilli are injected into the nonpregnant uterus of a cow, they disappear in the course of a few days. When the discharge from the uterus of a cow which has aborted is tested, abortion bacilli for 20, 30, or even 40 or 50 days may be found; but they eventually disappear, and it is the impression of the writers that their abundance and period of persistence are intimately related to the magnitude of the lesions in the uterus attendant upon an abortion.

It is the belief of the writers that the evidence they have supplied is sufficient to prove two facts: (1) that the udders of cows are a common habitat of abortion bacilli, and (2) that abortion bacilli do not maintain themselves in the bodies of nonpregnant cows elsewhere than in their udders. The occurrence of the bacilli in the supramammary glands, and in one instance in pelvic lymph glands, and no farther in the body, merely proves that the germs tend to penetrate into the body from the udder through the lymph channels, but that they can not go very far before they are destroyed.

PRODUCTION OF SEEMINGLY NORMAL CALVES BY INFECTED COWS

When abortion bacilli are injected into the udder through the teat, by a method which avoids a trauma, the bacilli are established in the udder, and the cow, according to all available tests, becomes an infected cow.

There is a remarkable and truly important fact concerning the production of calves by cows with infected udders. Such cows, irrespective of whether they have, at some time in the past, aborted or not, may give birth to seemingly normal calves in a seemingly normal manner associated with the occurrence of abortion bacilli in their uteri and in the afterbirth. Quite a number of records prove this, and although it does not occur every time a cow with an infected udder calves, it is far from uncommon. As has been stated, it may occur with a cow which has never aborted; and it may occur with the third seemingly normal parturition after an abortion. In the experience of the writers, in which they have made a number of tests, this remarkable fact has never been observed in connection with cows which react positively with the agglutination test but the udders of which were free from infection. And the fact becomes all the more remarkable when it is viewed in the light of another fact—namely, that numerous careful tests of the uteri of nonpregnant cows, irrespective of whether their udders were infected or not, tests made both between and during periods of oestrus, in no instance revealed the presence of abortion bacilli.

Another fact which merits consideration in this connection was derived from tests with newly born calves. A number of calves produced by cows with infected udders were killed immediately after they were born and their bodies tested for the presence of abortion bacilli through guinea-pig inoculation methods. These calves were not permitted to come into contact with their mothers or other sources of infection that would tend to introduce germs into their bodies not present at the moment of completed parturition. It was found that such calves—those that were delivered alive and seemingly vigorous and healthy—may harbor abortion bacilli in their stomachs and gastrohepatic lymph glands; but invariably, when the calves were infected, the afterbirth and the uteri of their dams were also infected. In aborted fetuses the stomachs, intestines, lymph glands, spleens, livers, blood, and subcutaneous extravasations of serum may contain abortion bacilli.

EXPERIMENTAL INFECTION INTRODUCED THROUGH TEAT

One record of the injection of abortion bacilli into the udder of a cow, through the teat without trauma, is particularly interesting. The cow was well advanced in pregnancy and, according to all tests that could be made, was free from abortion disease prior to the injection. This record is given in detail because it is very instructive and also illustrates the laborious application the investigation of abortion disease requires. In this connection it may be observed that in this work the writers have used the agglutination test rather than the complement-fixation test for abortion disease. The reason for this is that the writers are convinced that the agglutination test for this disease is fully as reliable as the complement-fixation test, but far less complex; hence, in the hands of those who have many and varied duties, it is more reliable.

RECORD OF COW 1154

September 9, 1914. Received at the experiment station from an abortion-free herd. About 8 years old. Was negative to all tests for abortion disease and was carefully protected against exposure to infection.

August 21, 1915. Served by bull 1150 and conceived. The bull was received at the station on the same day on which the cow was received, and was and is now negative to all tests for abortion disease, and has been carefully protected against exposure to infection.

December 10, 1915. Agglutination tests with blood serum from the cow and the bull were made. Negative in both cases.

March 27, 1916. Agglutination tests with blood serum from the cow and the bull were made. Negative in both cases.

March 27, 28, 29, 30, 31, 1916. Material was obtained on each day from the udder of the cow and injected into guinea pigs. The guinea pigs were subsequently killed and examined post mortem and found to be free from lesions of the kind caused in guinea pigs by abortion bacilli; in fact, they had remained perfectly healthy and showed no lesions of any kind.

April 3, 1916. The growth on two culture tubes of abortion bacilli was scraped off and suspended in 30 c. c. of sterile normal salt solution and injected into

the right front teat of the cow. The method of injection was through gravity, and the pressure used did not exceed that exerted by a column of fluid 12 inches high. Two guinea pigs were injected with samples of the suspension, and both later showed typical lesions of the kind caused in guinea pigs by abortion bacilli.

April 8, 1916. Five days after the injection agglutination tests with blood serum from the cow were negative.

April 17, 1916. Two weeks after the injection agglutination tests with blood serum from the cow were positive with dilutions of 1 to 400, which must be regarded as a very strong reaction.

April 22, 1916. Material from the infected quarter of the cow's udder was injected into guinea pigs, which subsequently developed typical abortion-bacillus lesions.

May 3, 1916. Material from each quarter of the cow's udder was injected separately into guinea pigs, all of which subsequently developed typical abortion-bacillus lesions, showing that the infection originally introduced into one quarter had spread to the other three quarters. On the same day material from the udder agglutinated suspensions of abortion bacilli in the following dilutions:

Right front, or injected quarter.....	1 to 6,400
Left front quarter.....	1 to 1,600
Right hind quarter.....	1 to 800
Left hind quarter.....	1 to 1,600

It is interesting to note how much higher the agglutinating value of material from the injected quarter is than from the other quarters. The material obtained from the cow's udder is not called "milk," because the cow was practically dry; and it is questionable whether the material which can be stripped from a practically dry udder shortly before parturition can reasonably be looked upon as milk.

May 9, 1916. The agglutinating value of material from the injected quarter of the udder was positive in a dilution of 1 to 12,800, and on May 15, 19, and 24, in a dilution of 1 to 25,600. On these days the agglutinating value for suspensions of abortion bacilli of material from the other quarters of the udder remained constant for a dilution of 1 to 1,600, and that of the blood serum of the cow for a dilution of 1 to 400.

May 26, 1916 (279 days after service by the bull). The cow produced an undersized, weak calf, which, however, rapidly gained strength and is now a normal, healthy, vigorous animal. On the day of parturition the following agglutination tests were made:

Colostrum, injected quarter of udder, positive, dilution 1 to 25,600.
Colostrum, other three-quarters of udder, positive, dilution 1 to 1,600.
Blood serum, cow, positive, dilution 1 to 400.
Blood serum, calf, positive, dilution 1 to 400.

When agglutination tests are made with blood serum, it is common for newly born calves of infected cows to react in the same dilutions or quite as strongly as their mothers, but this power to react does not persist; it is a rapidly declining phenomenon, as is well shown by the following tests of the blood serum of the calf concerned in this record.

On the day of its birth, as above recorded, the agglutination value of the calf's blood serum and that of its mother were identical; positive in dilutions of 1 to 400. Seven days later, June 2, the agglutination value of the calf's blood had declined to 1 to 200; on June 7 it had fallen to 1 to 100; on June 9 it was still at 1 to 100; but on July 10

all agglutinating power for suspensions of abortion bacilli had disappeared.

Contrary to this, the agglutinating power of the cow's blood serum remained constant for dilutions of 1 to 400. Not so, however, with the agglutinating power of material from her udder. Colostrum, as has been seen, agglutinated in dilutions, injected quarter, 1 to 25,600; other quarters 1 to 1,600. The milk as early as June 8, or 13 days after parturition, was positive in dilutions no higher than 1 to 200 in the injected quarter, and 1 to 50 in the other quarters, at which points it remained fairly constant.

The most interesting fact about this cow was that parturition was associated with retention of the afterbirth, which, on removal, was found to contain much abnormal material of a yellowish color, and this was proved to be infected with abortion bacilli. Vaginal discharge from the cow was also proved to be infected with abortion bacilli on June 1, 3, and 12, and free from infection on and after June 20.

THE UDDER AS A POSITIVE CHANNEL OF INFECTION

This one cow illustrates a number of abortion-disease phenomena. First, the introduction of abortion bacilli into the udder through the teat, though a method of injection was used which almost certainly precluded mechanical injury, positively infected it and caused the gradual development of agglutinating power for suspensions of abortion bacilli in the blood serum. In other words, the udder is a possible channel through which abortion bacilli may penetrate into the body.

Second, the passage of abortion bacilli from the udder to the uterus is an experimentally demonstrated fact. The writers have already stated that, in all cases in which they found abortion bacilli in the uterus after seemingly normal parturitions, the cows had infected udders; and it is only necessary to add that, in practically half of the cows with infected udders that have been examined relative to this matter, the uterus and placenta were infected with abortion bacilli.

It has been suggested that the abortion disease may perpetuate itself through abortion bacilli that enter the udder through the teat. When we consider how cows are milked, and how the milker goes from cow to cow without disinfecting his hands, and that the udders of cows are the commonest habitat of abortion bacilli, this mode of infection can not be regarded too lightly, or as an untenable supposition. That this is a means of perpetuation has not been proved, but it should be considered as a possibility.

In the third place, the record of cow 1154 illustrates another fact—namely, the high agglutinating power of colostrum from cows with infected udders. This phenomenon, together with the rapid decline of agglutinating power of material from the udder as milk takes the place of colostrum, has been repeatedly observed.

In the fourth place, the rapidly declining agglutinating power of the blood serum of the calf of an infected cow is shown, and this also is a repeatedly observed phenomenon. The writers have found that agglutinating properties can be engendered in the blood of calves by injecting them with abortion bacilli; but such injections must be repeated from time to time, otherwise the agglutinating properties of the blood serum disappear.

In the fifth place, as the calf was suckled by its mother, whose udder was known to be heavily infected, it may be judged from the rapidly declining agglutinating value of its blood that abortion bacilli in ingested milk do not seem to penetrate deeply or abundantly into a calf's body. The records of other cows and calves give similar data.

POSSIBILITY OF INFECTION THROUGH THE BULL

It is rare for male and virgin cattle to react positively to abortion tests, and it has been pointed out that the bodies of cows do not harbor abortion bacilli elsewhere than in their udders, associated lymph glands, and pregnant uteri. It does happen occasionally that bulls do react when they are tested for abortion disease, and what such reactions may signify remains decidedly questionable; hence, the two following cases may be both instructive and interesting.

Sometime ago the writers found two bulls which reacted when their blood serum was tested with suspensions of abortion bacilli. In one case the reaction was positive in a dilution of 1 to 200 and in the other in a dilution of 1 to 100. Where the bulls got the infection the knowledge of their history does not reveal.

One of the bulls, the one with the higher reaction, was immediately killed and examined. The only lesion found in his body was an abscess involving the epididymis of one testicle, and this abscess was definitely proved to be infected with abortion bacilli. Tests of all other portions of the sexual organs and various other organs of the body failed to reveal abortion bacilli.

Was this apparently healthy bull qualified to serve as an active disseminator of abortion disease? The writers are not ready to answer the question at present.

The other bull was permitted to serve a cow which, according to her history and all tests made, was free from abortion disease. Immediately after the service seminal fluid was recovered from her uterus and injected into a number of guinea pigs, one of which subsequently showed abortion bacillus lesions. Tests are still being carried on with this bull.

RELATION OF THE ABORTION BACILLUS TO THE EMBRYO OR FETUS

A few years ago one of the writers, on the basis of the work on abortion disease, expressed the view that the abortion bacillus seemed to have a peculiar affinity for embryonic tissue. They are still of this opinion, and

it is possible that the disease is in fact primarily a disease of the embryo or fetus rather than of its mother. The mother, to be sure, is the source of infection. Possibly, however, if a large enough number of virulent abortion bacilli are poured into her body from her udder, antibodies of sufficient potency may develop in her blood to protect her fetus. Should this prove true, good results in the treatment of infected herds may be expected from injections into the mother, possibly a short time before she conceives or early during pregnancy, of cultures of abortion bacilli; and it is possible in this case that the more virulent the cultures are and the more abundant the material injected the better the results will be.

CONCLUSION

To prevent the further spread of abortion disease, owners of uninfected cattle should be instructed to have careful agglutination tests for abortion disease made of all cattle they propose to introduce into their herds; and owners of infected herds should be taught that aborted fetuses, also the afterbirth and discharge from the vaginas of infected cows, are infected with abortion bacilli and must therefore be disposed of with care.

The treatment of individual cows which have aborted or failed to clean properly after parturition must be left largely to the good judgment of the practicing veterinarian. If the uterus is given a proper chance to heal after it has been damaged by an abortion or a retained afterbirth, the abortion bacilli in it need occasion little worry, as they will rapidly disappear of their own accord, and it is very questionable whether reparative processes are not retarded rather than facilitated by douching with germicidal solutions which are strong enough to kill bacteria in a reasonable length of time, or the length of time during which they may remain undiluted in the uterus. Douching is no doubt good practice, but it is desirable that there be a flooding out, a washing out, a real physical cleaning of the uterus; and this can best be done with solutions which are healing rather than germicidal, soothing and not irritating.

WHEAT-SHEATH MINER

By H. L. SEAMANS,

Assistant in Entomology, Montana Agricultural Experiment Station

INTRODUCTION

In an investigation in 1915 of wheat plants (*Triticum* spp.) supposed to be infested with *Meromyza americana* Fitch, two different forms of larvæ were noticed, one a greenish larva, apparently that of *M. americana*, and the other a smaller, whitish one. Further examination showed the latter to be more plentiful and that the damage to wheat plants supposed to be caused by the *M. americana* was mainly due to the other species. A few plants were taken to the insectary at Bozeman, Mont., and the insects on coming to maturity were found to be mainly *Cerodonta femoralis* (Meigen), a species about which very little has previously been known.

HISTORY OF THE SPECIES

This insect was first described by Meigen in 1838 (2, p. 397)¹ as *Agromyza femoralis*. At that time the genus *Agromyza* Fallen embraced a number of species now placed in genera since split off. In 1835, however, Macquart (1, p. 214) had used the name "Odontocera" to designate the genus which in 1861 Rondani (3, p. 10) called "Cerodonta;" but, "Odontocera" being preoccupied, it can not be used here. In 1862 Schiner (4 and 5) gave this genus the name "Ceratomyza," and this is the name now used by some authors. Later writers on this group of flies, Melander (7) and Malloch (8, p. 331), have used Rondani's "Cerodonta" for this genus, which has priority over "Ceratomyza"; therefore Meigen's *Agromyza femoralis* then becomes *Cerodonta femoralis*. A search of the literature reveals practically nothing about the biology of this insect. Neither Aldrich nor Malloch discusses it, although Aldrich includes a note under the genus *Ceratomyza*, which reads:

An undescribed *Ceratomyza* has been reared from young wheat plants at Pullman, Wash., by Prof. C. V. Piper; it causes considerable damage.

According to recent information from Melander, who is now Entomologist at the Washington Agricultural Experiment Station, this fly was *C. femoralis*. Melander (8) mentions the species only in a key to the genus *Cerodonta*.

DISTRIBUTION OF THE INSECT

Melander (8) reports having specimens from Europe, Montana, Wyoming, Idaho, Washington, Oregon, California, and British Columbia. This would indicate that it is generally distributed in the Northwest.

¹ Reference is made by number to "Literature cited," p. 24-25.

In Montana injury has been reported, and specimens have been received in material from the following towns: Arlee, Ronan, Twin Bridges, Brady, and Seventynine. This covers pretty well the whole western portion of the State, though perhaps the most severe damage has been done at Arlee. Most of the material for this work was collected there.

HOST PLANTS

Specimens of *C. femoralis* were found in or reared from winter wheat, spring wheat, oats (*Avena sativa*), and timothy (*Phleum pratense*). The latter was found growing abundantly along the ditches and fences surrounding the field. Volunteer wheat and oats were also found to be infested during the latter part of September. As this insect has been found only in plants belonging to the Graminae, it appears that native grasses may be its natural host plant.

DESCRIPTIONS OF THE LIFE STAGES

EGG

The egg is ellipsoidal, white, soft, smooth, translucent, glistening, 0.5 mm. long, and from 0.15 to 0.2 mm. broad.

LARVA

The larva is white tinged with green, excepting for the blackish or brownish alimentary canal, which shows through the body wall. It is about 3.0 mm. long and 0.5 mm. in diameter when full grown. There are 13 segments. The mouth parts are indistinct, but are represented by black, chitinous hooks. There are two tubercles on the median line at the anterior end on the ventral surface, one each on the second and third segments. The first three and the last segments are short, the rest being of equal length, are slightly wider than long. The oral opening is found on the ventral side of the first segment and the anal opening on the ventral side of the last segment.

There are two pairs of respiratory organs, or breathing appendages, one at each end of the body. They are similar in structure, and those on the same side are connected by a tracheal tube, which is visible through the larval skin as a fine, white thread. Each appendage consists of a main stalk which branches into five smaller stalks, at almost right angles. Each of these small stalks has from one to three openings, the total number varying from 8 to 11.

The anterior segment of the body bears a patch of short black spines, placed just above the mouth hooks.

PUPARIUM AND PUPA

The puparium is moderately chitinated, brownish, semitransparent, 3.0 mm. long and 1.25 mm. broad in the middle, slightly tapering toward the ends and somewhat flattened dorsoventrally. There are 10 plainly visible segments, the anterior and posterior ones being hemispherical.

Toward the end of the pupal period the pupa is visible through the puparium, showing the parts of the body distinctly, and movements in the legs can be seen three days before the emergence of the adult fly.

ADULT

A translation of Meigen's description (2, p. 398) of the adult fly is as follows:

Black; head, pleurae, and femora yellow; third antennal joint black, with distal thorn. Head light yellow, with black ocellar spot. Base of antennae yellow, third joint deep black, with black arista and apical spine. Notum and scutellum shining black, pleurae yellow, abdomen black and shining. Femora yellow, tibiae and tarsi piceous. Halteres white; wings grayish. $2\frac{1}{3}$ line.

From an abundance of material of both sexes the following description has been prepared:

Cerodonta femoralis (Meigen).

Adult male: Length, 2.0 to 2.5 mm.; wing expanse, 4.0 mm.

Head as broad as the thorax; front broad, about one-third the width of the head, yellow except for a rectangular, black, ocellar spot at the vertex. Three pairs of frontal bristles present, reaching to the base of the antennae; one pair of ocellar bristles directed forward, situated at the two anterior corners of the ocellar spot; two pairs of vertical cephalic bristles, the outer pair divergent, the inner pair convergent; one pair divergent post vertical bristles present, located at the posterior margin of the ocellar spot. The three ocelli are located, one in the middle of the anterior margin, and the others in the middle of each of the lateral margins of the ocellar spot; pitillum visible as a raised triangular portion, just above the base of the antennae. Oral vibrissae present, with four pairs of small hairs along the oral margin. Oral region, including the labella and genae, bright yellow.

Antennae three-jointed, the first joint small, ringlike, and yellow; second joint larger, brown in color, with one medium-sized bristle, and a coronet of small bristles; third joint the largest, black oval, with a dorsal arista and an apical spur, pubescent.

Occiput black; postorbital bristles present.

Thorax for the most part shining black; pleural sclerites yellowish, or gray bordered with yellow. Four pairs of dorso central, one pair each of humeral, posthumeral, notopleural, supraalar, postalar, and scutellar apical bristles present. One propleural bristle, and one large and from two to four small mesopleural bristles present; sternopleurite with one bristle below the sternopleural suture, and a fanlike row of bristles above the middle coxa.

Anterior coxae large, femora bowed; all coxae with bristles; femora with a small bristle on the posterior surface; anterior tibia alone without apical spine; coxae and femora yellow, tibia and tarsi brown.

Wings grayish, basal portions of veins yellow, darker toward the tip; halteres whitish or yellow; squamae small, colorless.

Abdomen viewed dorsally with six easily distinguished segments (the sixth is a genital segment) tapering posteriorly, uniformly hairy, black, except for a narrow yellowish line on the posterior margin of the first two and sometimes all of the segments. The fifth segment is about twice as long as the preceding; the sixth is somewhat globular in shape, with the posterior and ventral surfaces cleft, so that the two lateral plates form a pair of claspers. A chitinous process may protrude through the posterior cleft, and the penis hinges on this process, being directed forward. The dorsal surface of the penis near the hinge is open and the vas deferens, a flexible tube, follows along the chitinous process and enters the penis at the dorsal opening. The chitinous process upon which the penis is hinged appears to extend to the second

segment. When not protruded, the chitinous process is drawn up into the body, sheathing the penis within the ventral portion of the fifth segment.

The size of the female is the same as that of the male, and in general description the two sexes are alike. Abdomen less tapering, six segments, a seventh segment sometimes visible when the ovipositor is protruded. Ovipositor retractile, tubular, with a hard, chitinous edge. Posterior segment not cleft ventrally.

NATURE AND EXTENT OF INJURY

A field of wheat infested with the wheat-sheath miner may not appear to be greatly injured. Unless the field is badly infested the grain has a

healthy color and appears to be strong. A badly infested field appears slightly off color and may show areas which look decidedly unhealthy. It is necessary to make a close examination of individual stools to even begin to estimate the real damage. This is true especially of winter wheat, where many of the stems of the fall growth may be present only as dried and withered leaves.

The injured culms are instantly recognized by the fact that, while the leaves, or at least part of them, are green and apparently healthy, the central stalk is dead and withered (fig. 1). The injury done by *C. femoralis* appears identical

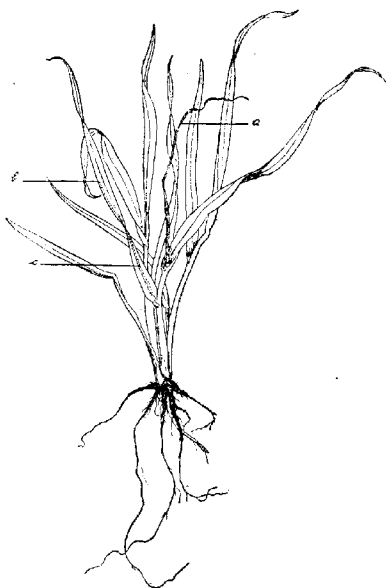


FIG. 1.—Young wheat plant, showing (a) central stalk injured by the wheat-sheath miner, *Cerodonta femoralis*; (b) point where egg was laid; (c) mine in the leaf made by larva on its way to the sheath; other leaves normal.

with that of *Meromyza americana* until the leaves are examined. In each case the larva enters the leaf sheath by mining down from the point in the leaf where the egg was laid. If the injury is due to *C. femoralis*, the mine in the leaf is narrow, clean-cut, and almost straight; while if it is done by *M. americana* it is broad and irregular with indistinct edges. While *M. americana* enters the stem and eats out the central stalk, usually cutting it off above the first node, *C. femoralis* confines its attack to

mining up and down the leaf sheath and sometimes girdling the stem without completely cutting it off. Whether it girdles the stem or not, the injuries caused by mining in the sheath appear to be sufficient to kill the stalk.

Some of the farmers who were interviewed in regard to the damage done by this insect estimated that their yields of winter wheat had been cut down at least 25 per cent in the season of 1915. Though new shoots often spring up from the lower nodes after the central stalk is killed, these are not as strong and do not bear as large or as full heads of grain as the normal stalk.

A field of spring wheat was visited in 1916, in which by actual count 95 per cent of the plants showed injury by this insect. Some of these plants had only one culm injured, while others had lost two or three. An adjoining field of oats was found to have 12 per cent of its plants infested.

There is a slight amount of injury done to the plants, just before blossoming, by the second brood of larvæ. This injury is only to the leaves and probably has little or no effect on the yield, as the central stalk does not seem to be injured.

To what extent the larvæ injure winter wheat in the fall has not yet been ascertained. Farmers have stated that they have seen the injury in winter wheat, but it has been impossible to make an investigation of this statement because very little winter wheat has been grown in the infested localities in the last two years.

LIFE HISTORY AND HABITS OF THE MINER

The study of the life history of the wheat-sheath miner was carried on in the insectary at Bozeman, with material collected in the vicinity of Arlee. Several plants containing eggs and larvæ were collected at Arlee on June 7, 1915, and were brought in for the purpose of rearing a supply of adults and securing all the data possible regarding this species.

Adults emerged in the insectary on July 11 and continued to emerge until July 24. These adults were kept alive for some time, and their habits were studied in various ways. The first flies to emerge were placed in lamp-chimney cages over seedling wheat plants. The females fed on the juices of the wheat plants by making tiny incisions in the upper surface of the leaves and then lapping up the juices which exuded. One female fly caged over a wheat plant made 102 feeding punctures in 24 hours. The males showed no inclination to feed on these juices; but when some wheat blossoms were placed in the cages they appeared to feed on the pollen, touching the anthers continually with the labella. The flies began feeding almost immediately on emerging, and one newly emerged female was seen to make three feeding punctures in five minutes.

Copulation may take place any time after 24 hours from emergence, and this is followed by a preoviposition period of about three days. The oviposition period in the insectary was about 10 days, but is, without doubt, longer under natural conditions.

The plants which were brought in from the field on June 7 yielded flies from July 11 to July 24. As there was no possibility of eggs being laid after June 7 in those particular plants, theoretically the last eggs laid produced flies that emerged on July 24. If all the flies completed their life cycle relatively in the same length of time, the eggs which produced the flies emerging on July 11 must have been laid 13 days earlier than June 7, or on May 25. As the flies were plentiful on June 7 and were still laying eggs, it is probable that the oviposition period lasts about three weeks, or from May 20 to June 10.

The number of eggs laid by a single female was not ascertained, though six flies, caged over wheat plants, laid 96 eggs in 24 hours, which gives an average of 16 eggs per fly for that time.

The egg-laying process is simple and takes from 7 to 12 seconds. The ovipositor is brought at right angles to the surface of the leaf, the upper epidermis is punctured, and the ovipositor is forced underneath it, toward the base of the leaf. A contraction of the abdomen forces the egg into the lower end of the puncture and the ovipositor is withdrawn, leaving the egg well protected under the epidermis. The feeding punctures are made in a similar manner, except that the leaf surface is scraped instead of the epidermis being punctured.

The incubation period of the eggs is about six days under insectary conditions. Eggs were laid in wheat plants on two consecutive days, fresh plants being substituted each day. These eggs were watched, and six days later the first ones hatched and were followed by the rest the next day.

Immediately on hatching from the egg the larva starts mining down the leaf toward the stalk, eventually ending in the leaf sheath, at the crown of the plant, or at the first node. On reaching the base of the leaf sheath the larva feeds up and down the sheath, and sometimes around the stalk.

The length of the larval period is variable, as some of the larvæ pupated at the end of 10 days, while some took as long as 20 days, depending on weather conditions. Cool, wet weather seemed to delay pupation.

Some of the larvæ were removed from the leaf sheath and allowed to pupate in a tin box. Others were left in the plants to pupate normally. They did not leave the sheath, but pupated either at the node or down close to the crown of the plant. None of them entered the soil or worked into the center of the stalk for pupation.

The pupal period lasts about 25 days under insectary conditions. Larvæ pupated in plants and in tin boxes on June 22 and emerged on July 16 and 17. What the variation in the length of the pupal period would be under varying weather conditions can not be estimated. As an indication that insectary conditions were parallel with natural conditions, wheat plants that were sent in from the field on the same date contained pupæ from which flies were emerging.

SEASONAL HISTORY

There is some doubt as to the number of broods of the wheat-sheath miner in a year, though there seem to be three full broods, with the hibernation spent in the pupal stage.

The adult flies appear about May 20 and lay eggs in wheat seedlings until about June 10. On June 16, 1916, no flies were caught over a badly infested field of wheat, though the day was ideal for making a collection. The flies were apparently through ovipositing and had disappeared by that time.

The flies of the second brood emerged in the insectary about July 16, and lived until August 5. As pupæ were sent in from the field about that time, from which flies were emerging, it appears that the second brood is present in the fields from about July 15 to August 10. This brood lays eggs in the leaves of the wheat and also in seedling volunteer wheat and grasses. The life cycle of this brood was carried out in the insectary, and the third generation of flies began to emerge September 7. By September 10 all the pupæ in the cages had emerged and the flies were put out of doors with wheat plants, to keep them under more normal conditions. They did not survive a sudden cold snap which occurred at Bozeman on September 13.

The next generation, which is believed to hibernate as pupæ and produce the first brood of flies the following spring, has never been reared in the insectary; and it is only by means of a few scattering facts that the occurrence of this generation is suspected.

Many farmers who were questioned concerning this insect told the writer that they had often found "a small, brown, wrinkled egg down next to the killed stalk in the winter wheat during the fall and winter." By this they doubtless meant that they had noticed the pupa in the dead stalk. Some of these men had noticed the same thing in the stubble after cutting in August. Since winter wheat is planted after the middle of August and the second brood of flies is apparently through ovipositing before the wheat has come up, the pupæ in the winter wheat must have been the result of eggs laid by the third brood of flies. During March, samples of winter wheat were sent in which contained pupæ apparently identical with those of *C. femoralis*. These pupæ never emerged, and the identity of the flies could not be definitely determined, though there is little doubt of their being *C. femoralis*. During the last week in September, 1916, a visit was made to the badly infested locality. Pupæ and fully grown larvæ were found in timothy, volunteer oats, and wheat. These were apparently the third brood and indicate the pupal hibernation, thus assuring three broods for the year.

It does not seem likely that the pupæ of the second brood would last through the fall and winter, since all those in the insectary had emerged by September 10. Neither does it seem likely that the adults would over-

winter and lay eggs in the spring, since those which emerged were killed by a sudden cold snap. While the climate at Arlee is warmer than that at Bozeman, the winter there is considerably colder than a September cold snap at Bozeman and more liable to be injurious to insect life.

PARASITES OF THE MINER

Two hymenopterous parasites were reared from the puparia of *C. femoralis*. The puparia which are parasitized are of a darker color, with the segmentations more distinct than in normal puparia. These parasites were determined by Mr. A. B. Gahan, of the Bureau of Entomology, through the courtesy of Dr. L. O. Howard, to be a new species of *Dacnusa* (Braconidae) and *Cyrtogaster occidentalis* (Chalcididae). There were not enough of either of these species to be effective agents in control.

CONTROL

No control measures have as yet been tried, but a knowledge of the seasonal history of the fly leads to suggestions. At the time wheat is cut for harvest, the larvæ of the second brood are in about the last instar at the crown of the plant, or have already pupated. Scattering the straw over the field and burning the stubble as well as grass borders surrounding the fields would doubtless get rid of a high percentage of the flies. Should this insect ever become a very important factor in grain growing, it is possible that it would be desirable to use a header or cut the grain very high, either of which would leave the straw on the field where it could be burned. However, if burning the stubble is not practicable, plowing it under about 6 inches and harrowing just after removing the crop or before planting a spring crop would probably bury the pupæ deep enough to prevent the flies from emerging.

The late seeding of winter wheat, after a thorough destruction of volunteer grain and grass, would doubtless accomplish much in control. The wheat could be sown about the third week in September, and would not be up until after the greater number of flies had finished ovipositing. This would not only prevent the injury to the wheat itself, but would do away with the main source of infestation in the spring.

As native grasses are probably the natural hosts of the fly, crop rotation would be almost useless. However, the cleaning up of field borders and destruction of volunteer stools during the fallowing period would aid greatly in the control of this insect.

LITERATURE CITED

- (1) MACQUART, Justin.
1835. Histoire Naturelle des Insectes Diptères. v. 2. Paris.
- (2) MEIGEN, J. W.
1838. Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten. T. 7. Hamm.
- (3) RONDANI, Camillo.
1861. Dipterologie Italica Prodrum. v. 4. Parmæ.

- (4) SCHINER, I. R.
1862. Vorläufiger Commentar zum dipterologischen Theile der "Fauna austriaca". In *Wien. Ent. Monatschr.*, Bd. 6, p. 428-436.
- (5) ———
1864. *Fauna Austriaca; Die Fliegen (Diptera)*. Bd. 2. Wien.
- (6) ALDRICH, J. M.
1905. Catalogue of North American Diptera (or two winged flies). 680 p. Washington, D. C. (Smithsn. Misc. Collect. pt. of v. 46.)
- (7) MELANDER, A. L.
1913. A synopsis of the dipterous groups Agromyzinae, Milichiinae, Ochthophilinae and Geomyzinae. In *Jour. N. Y. Ent. Soc.*, v. 21, no. 3, p. 219-273; no. 4, p. 283-300, pl. 8.
- (8) MALLOCH, J. R.
1913. Revision of the species of *Agromyza* Fallen, and *Cerodontha* Rondani. In *Ann. Ent. Soc. Amer.*, v. 6, no. 3, p. 269-336, pl. 28-31.